REMARKS

Reconsideration of this application, as amended, is respectfully requested. Originally filed claims 1-39 have been cancelled and new claims 40-59 inserted. As the claims have been amended, the fees due with this response differ from those listed on the Notice. Nineteen total claims, with three independent claims are now pending. As such, the additional claims fees listed in the Notice are not required. The specification has been amended by inserting SEQ ID NOS. and the new sequence listing. These amendments do not add any new matter. Applicants reserve the right to prosecute and cancelled or unclaimed subject matter in a subsequent continuation or divisional application.

Sequence Listing

A new Sequence Listing is attached in both paper and computer-readable form (2 CD-ROMs). The undersigned hereby declares that the content of the paper and computer readable copies of the Sequence Listing submitted herewith are identical in content and include no new matter. The specification has been amended to insert the Sequence Listing.

CONCLUSIONS

Reconsideration of this application, as amended, is respectfully requested. Applicants believe the claims are now in condition for allowance, and respectfully request that the Examiner issue a Notice of Allowance as soon as possible. If the Examiner believes it would expedite prosecution of this application, he is encouraged to contact the undersigned at his convenience.

09/06/2007 MKAYPAGH 00000114 500244 10584378

01 FC:1642 400.00 OP
02 FC:1633 200.00 OP
03 FC:1617 130.00 OP
04 FC:1616 360.00 OP
05 FC:1615 20.00 DA 1230.00 OP
06 FC:1614 500.00 DA

Date: September 4, 2007

Respectfully submitted,

Patrick J. Halloran

Reg. No. 41, 053

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variant thereof. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically from about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein. Suitable fusion segments include, among others, metal binding domains (e.g., a poly-histidine segment), immunoglobulin binding domains (i.e., Protein A, Protein G. T cell, I B cell, Fc receptor, or complement protein antibody- binding domains), sugar binding domains (e.g., a maltose binding domain), and/or a "tag" domain (i.e., at least a portion of α-galactosidase, a strep tag peptide, a T7 tag peptide, a FLAG peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies).

This tag is typically fused to the polypeptide upon expression of the polypeptide, and can serve as a means for affinity purification of the sequence of interest polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified sequence of interest polypeptide by various means such as using certain peptidases for cleavage. As described below, fusions may also be made between a TA and a co-stimulatory components such as the chemokines CXC10 (IP-10), CCL7 (MCP-3), or CCL5 (RANTES), for example.

A fusion motif may enhance transport of an immunogenic target to an MHC processing compartment, such as the endoplasmic reticulum. These sequences, referred to as tranduction or transcytosis sequences, include sequences derived from HIV tat (see Kim et al. 1997 J. Immunol. 159:1666), Drosophila antennapedia (see Schutze-Redelmeier et al. 1996 J. Immunol. 157:650), or human period-1 protein PER1; in particular, SRRHHCRSKAKRSRHH; SEQ ID NO.: 1)

In addition, the polypeptide or variant thereof may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to: an epitope to allow for the detection and/or isolation of a fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an

variant thereof. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically from about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein. Suitable fusion segments include, among others, metal binding domains (e.g., a poly-histidine segment), immunoglobulin binding domains (i.e., Protein A, Protein G. T cell, I B cell, Fc receptor, or complement protein antibody- binding domains), sugar binding domains (e.g., a maltose binding domain), and/or a "tag" domain (i.e., at least a portion of α-galactosidase, a strep tag peptide, a T7 tag peptide, a FLAG peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies).

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EXAMPLES

Example 1

Vectors

A. Construction of the Multi-Antigen Construct vcp2086

An expression vector was constructed in the ALVAC(2) vector using standard techniques. DNA sequences encoding LFA-3 (Wallner, et al. (1987) J. Exp. Med. 166:923 932), ICAM-1 (Staunton, et al. (1988) Cell 52:925- 933) and B7.1 (Chen, et al. (1992) Cell 71:1093-1102) were inserted into the C3 locus of ALVAC. LFA-3, ICAM-1 and B7.1 form an expression cassette known as TRICOM. DNA sequences encoding CEA-CAP1(6D) and p53 were inserted into the ALVAC donor plasmid pNC5LSPCEAp53 as shown in **Figure 1** (SEQ ID NOS.: 2 and 3). This donor plasmid was then used with the ALVAC-TRICOM vector to generate vcp2086 (ALVAC-CEA-p53-TRICOM).

B. Construction of the Multi-Antigen Construct Containing CEA-CAP1- 6D-1,2

An expression vector is constructed in the ALVAC(2) vector using standard techniques. DNA sequences encoding LFA-3 (Wallner, et al. (1987) J. Exp. Med. 166:923- 1 932), ICAM-1 (Staunton, et al. (1988) Cell 52:925- 933) and B7.1 (Chen, et al. (1992) Cell 71: 1093-1102) are inserted into the C3 locus of ALVAC. LFA-3, ICAM-1 and B7.1 form an expression cassette known as TRICOM. DNA sequences encoding CEA-CAP1(6D)- 1,2 (**Fig. 2**; SEQ ID NO.: 4) and p53 are inserted into the ALVAC donor plasmid essentially as shown in Figure 1. In this vector, CEA-CAP1-6D (SEQ ID NO.: 5) is removed and CEA-CAP1-6D-1,2 (**Fig. 2**) is inserted using standard techniques. This donor plasmid was then used with the ALVAC-TRICOM vector to generate vcp2086 (ALVAC-CEA-p53-TRICOM).

EXAMPLE 2

Immunogenicity of Multiantigen Vectors

This series of experiments was designed to confirm the immunogenicity of the multiantigen expression vectors. As an example, vcp2086 was administered to the double transgenic mouse strain "CEA/A2K^{b}dbTg". These mice express both the chimeric HLA. A2kb Class I molecule as well as the human CEA gene as a "self" antigen. The potential to generate strong immunogenicity in this model depends upon the ability of the expression vectors to break tolerance and generate a T cell response to the self antigen CEA.

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Detection of anti-p53 responses is evaluated in the context of p53 being a foreign antigen, and therefore the issue of tolerance may not apply to p53 in this model.

A. Study MAD68

This experiment was designed as a dose titer of the multiantigen constructs. As a vector control, animals were immunized with the ALVAC(2) parental vector over an identical dose range. Analysis of immunogenicity is based on an ELIPSOT assay to detect IFN-γ production by peptide-specific T cells present in cultures from individual CEAxHLA. A2Kb Tg mice immunized with the indicated recombinant viruses. Groups of three individual mice were tested for each recombinant at a particular dose. Replicate cultures for all data points were tested against a control peptide to determine background response levels of the ELISPOT assay. The average of the three individual mice in each group was determined for comparison between groups. As a positive control, each individual culture group was tested using the mitogens PMA/ionomycin to induce IFN- from total spleen cells.

Individual spleen cells from the different groups (vcp2086 or ALVAC(2) parental vector at $1x10^8$; $2x10^7$; $2x10^6$; $2x10^5$ pfu/mouse) were harvested and re-stimulated in vitro with CEA or p53 peptides (**Table III**).

TABLE III

CEA and p53 Peptides

Peptide	Internal ID	Amino Acid Sequence	SEQ ID NO.
CEA-24	3205	LLTFWNPPT	6
CEA-233	1815	VLYGPDAPTI	7
CEA-691	571	IMIGVLVGV	8
CEA-78	3209	QIIGYVIGT	9
P53-139-147	3211	KTCPVQLWV	10
P53-149-157	3213	STPPPGTRV	11
P53-101-111	3215	KTYQGSYGFRL	12
P53-216	3217	VVVPYEPPEV	13

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U.S. Pat. No. 5,348,887). A modified version of KSA was synthesized in order to increase the capacity of the antigen to generate an immune response by, for example, increasing the ability of KSA to bind MHC molecules. KSA may be modified by changing any of several amino acids to effect the desired change in the antigen. The sequences of the wild-type KSA (GenBank M33011, Szala, et al. PNAS 87:3542-3546; SEQ ID NO.:14) and KSA containing a particular modification (SEQ ID NO.: 15) utilized herein are aligned in Figure 3 (sequence 1 represents M33011; sequence 2 represents the modified sequence, the modified sequences are indicated by an underline). In this manner, the T-cell epitope QLDPKFTTSI (175-184) (SEQ ID NO.:16) was converted to QLDPKFITSV (SEQ ID NO.: 17). Synthesis of the modified KSA sequence is described below.

B. Expression Constructs

The cDNA clone in plasmid pRW971 encoding the GA733-2 carcinoma-associated antigen (KSA) was obtained from A. Limenbach, The Wistar Institute, Philadelphia, PA. A XmaI-Spe I fragment containing the H6 promoter-KSA sequence was isolated from pRW971 and inserted into XmaI-SpeI sites on pBluescript to generate pBlu-KSA-l(R) (**Figure 4A**). To convert the codon ATT (He) at aa 184 of KSA to codon GTG (Vat), the pBlu-KSA-1 was subjected to mutagenesis using a Stratagene kit and primers 8109 (CAAAATTTATCACGAGT(GTG)TTGTATGAGAATAATG) (SEQ ID NO.: 18) and 8110 (CATTATTCTCATACAA(CAC)ACTCGTGATAAATTTTG) (SEQ ID NO.:19). The resulted plasmid mutant was designated pBlue-KSA-Val # 1 (SEQ ID NO.:20; **Figure 4A**). A XmaI-SpeI fragment was isolated from pBlue-KSA-Val #1 and inserted into the XmaI-SpeI sites on pT2255 generating pT2255 KSAV-1 (**Figure 4B**). A detailed plasmid map DNA sequence of pT2255-KSAV-1 are shown in **Figures 5A** and **B** (SEQ ID NOS.: 21 and 22), respectively.

The cDNA encoding LFA-3 was isolated at the National Cancer Institute by PCR amplification of Human Spleen Quick-Clone cDNA (Clontech Inc.) using the published sequence (Wallner et al. J. Exp. Med. 166:923- 932, 1987). The cDNA encoding ICAM-1 was isolated at the National Cancer Institute by PCR amplification of cDNA reverse-transcribed from RNA from an Epstein- Barr Virus-transformed B cell line derived from a healthy male, using the published sequence (Staunton et al. Cell 52:925-933, 1988). The cDNA encoding B7.1 was isolated at the National Cancer Institute by PCR amplification of cDNA derived from RNA from the human Raji cell line (ATCC CCL 86), using the published sequence (Chen et al. Cell 71:1093- 1102, 1992).

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